Etiologies of Premature ovarian failure (POF)

Pr Philippe Bouchard
Endocrinology
Hôpital Saint-Antoine
Université Pierre et Marie Curie, EA 1533 Paris

UNIVERSITE PIERRE & MARIE CURIE

Premature Ovarian Failure/ Insufficiency (POF/POI)

- Amenorrhoea > 6 months
- FSH > 40 mIU/ml
- Arbitrarily < Age 40

- 1% of all women

Goswami D Hum Reprod Update 2005

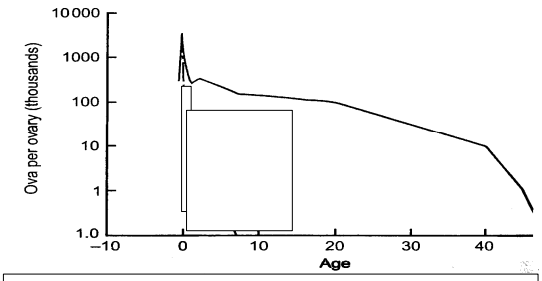
Premature Ovarian Insufficiency

Disease name and synonyms
Premature ovarian failure (POF; POF1: OMIM 311360); Hypergonadotropic ovarian failure; Menopausa precoce.

Included diseases
POF2 (OMIM #300511); POF3 (OMIM #608996)

Definition
Premature ovarian failure is defined as a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles/ arrested folliculogenisis before the age of 40 years (secondary amenorrhea).

Ovarian Follicular loss



The factors setting the age of menopause

- Environmental factors :
  Women who smoke reach menopause 2 years earlier than non smokers

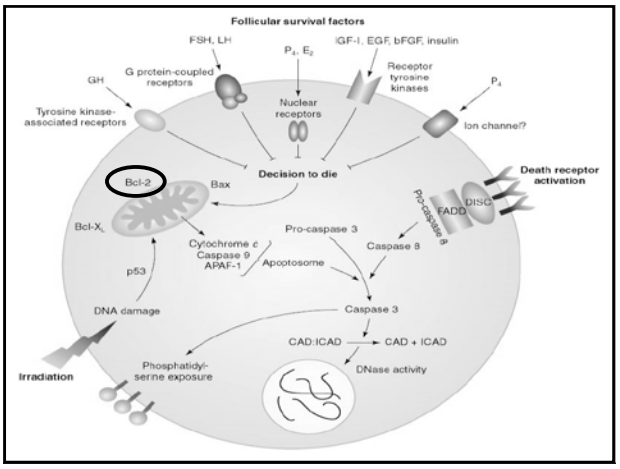
Midgette 1990 Epidemiology 1; 1479-480
Bromberger 1997 Am J Epidemiol 145; 124-133 Gold 2001 Am J Epidemiol 15; 634-639

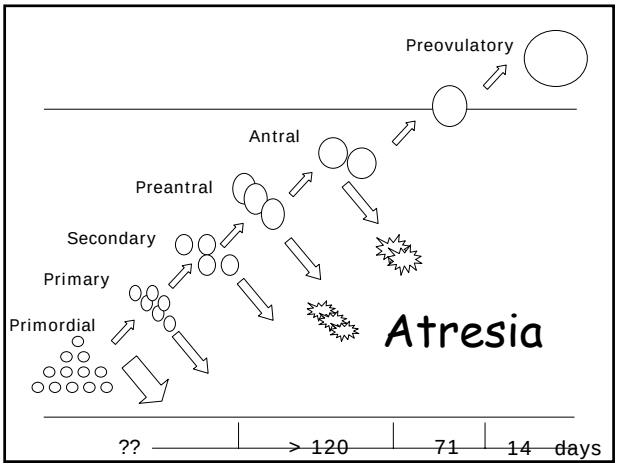
The factors setting the age of menopause

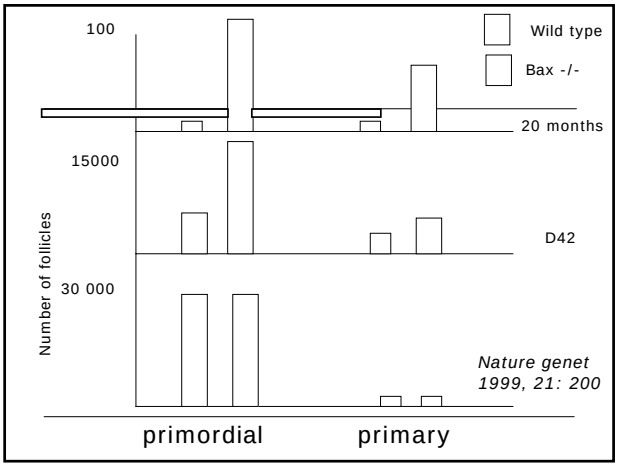
- Environmental factors :
  Animal models of ovarian failure

AhR -/- mice
Dioxin/aryl hydrocarbon receptor knock-out mice

At birth Twice the number of primordial follicles as compared to controls
Robles R Endocrinology 2000; 141: 450


Follicular survival factors
FSH, LH
IGF-I, EGF, bFGF, insulin
P_4, E_2
GH
G protein-coupled receptors
Receptor tyrosine kinases
P_4
Tyrosine kinase-associated receptors
Nuclear receptors
Ion channel?
Decision to die
Death receptor activation
Bcl-2
Bax
Bcl-X_L
FADD
DISC
Pro-caspase 8
Pro-caspase 3
Caspase 8
Cytochrome c
Caspase 9
APAF-1
Apoptosome
p53
DNA damage
Caspase 3
CAD:ICAD
CAD + ICAD
DNase activity
Irradiation
Phosphatidyl-serine exposure


Preovulatory
Antral
Preantral
Secondary
Primary
Primordial
Atresia
??
> 120
71
14
days


100
Wild type
Bax -/-
20 months
15000
D42
30 000
Number of follicles
Nature genet
1999, 21: 200
primordial
primary

POI

- Identify women/ families with POI and risk of transmission of a genetic disorder

POF

Etiology unknown
in more than 90% of cases,
apart from
surgery
chemotherapy, radiotherapy
and Turner syndrome

Genes and POF

Familial cases

In 15 - 20% of cases

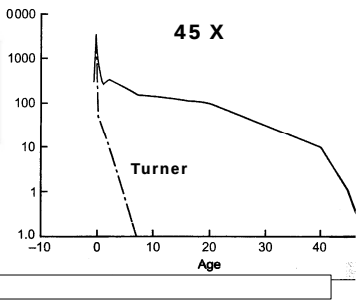
Heterogeneous Origin

- Iatrogenic origin (surgery, chemotherapy, radiations);
- Autoimmune, including polyglandular autoimmune syndrome, as well as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) due to mutations in *AIRE* gene);
- Infections (*e.g.* herpes zoster, cytomegalovirus);
- Chromosome X defects:
  - Turner syndrome
  - Fragile X syndrome (*FMR1* gene premutation)
- Monogenic defects
  - Syndromic defects:
    - Congenital disorders of glycosylation (CDG, former named carbohydrate-deficient glycoprotein syndrome (recessive)
    - Galactosemia (recessive)
    - Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (female-limited, dominant)
    - Pseudohypoparathyroidism (PHP) type Ia (parental imprinting : maternal inheritance)
  - Isolated defects:
    - Follicle stimulating hormone (FSH) receptor mutations (*FSHR*), (recessive)
    - Luteinizing hormone (LH) receptor mutations (*LHR*), (recessive)
    - *FOXL2* (transcription factor involved in BPES) mutations (female-limited defect, dominant)
    - Bone morphogenetic protein 15 (*BMP15*) mutations (female-limited defect, heterozygous mutation)
- Idiopathic

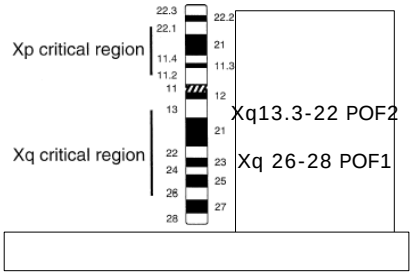
Table 1. Genes implicated in POF

Categories	Chromosome	Gene	Gene locus
Mutations identified	X chromosome genes	*FMR1*	Xq27.3
		FMR2	Xq28
		BMP15	Xp11.2
	Autosomal genes	*FOXL2*	3q22–q23
		FSHR	2p21–p16
		LH receptor	2p21
		FSH beta variant	11p13
		LH beta	19q13.32
		Inhibin A	2q33–q36
		GALT	9p13
		AIRE	21q22.3
		EIF2B2, -4, and -5	14q24.3, 2p23.3, 3q27
		NOGGIN	17q22
		POLG	15q25
Candidate genes	X chromosome genes	*DIAPH2*	Xq22
		DFFRX	Xp11.4
		XPNPEP2	Xq25
		ZFX	Xp22.3–p21.3
		FSHPRH1	Xq22
		XIST	Xq13.2
	Autosomal genes	*WT1*	11p13
		ATM	11q22.3
Mutations not identified	X chromosome genes	*AT2*	Xq22–q23
		c-kit	4q12
		SOX3	Xq26–q27
	Autosomal genes	*MIS*	19p13.3–13.2

Early follicular loss : Turner



X Chromosome

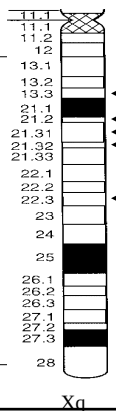


POF patients without clinical Turner phenotype

- Saint-Antoine, Lille, A Béclère
- 354 patients
- 40 control women

PHRC A0R1066

X, autosome translocations and Xq deletions

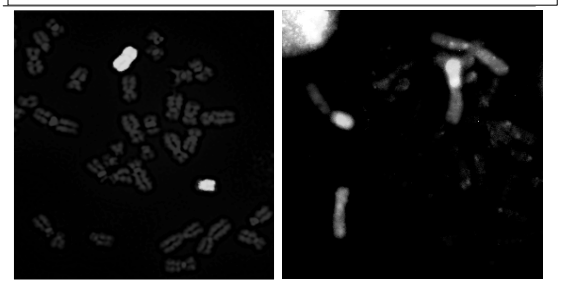


Primary amenorrhea

Amenorrhea age 25

Whole X painting

Whole X painting (green)
chromosome 1 (red)
(X;1) translocation



MF Portnoï et al Hum Reprod 2006

X chromosome study

- Characterisation of the translocation breakpoints

breakpoints fall in POF2
In poorly transcribed regions

=> Not an X-linked gene interruption

⇒ Position effect

on flanking X-linked genes
or genes flanking the autosomal breakpoints

X Chromosome

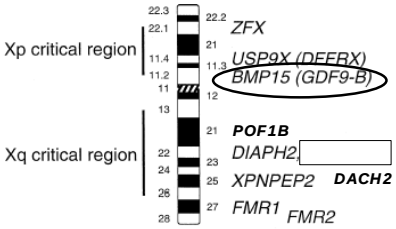


Figure 1. POF critical regions and candidate genes on the human X chromosome.

0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(5):1873–1876
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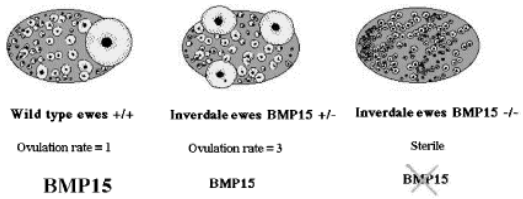
doi: 10.1210/jc.2003-030168

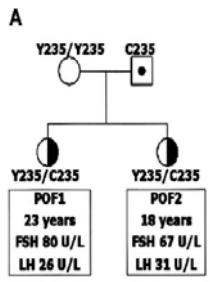
Editorial: BMP15—The First True Ovarian Determinant Gene on the X-Chromosome?

L Layman

BMP15 : Inverdale ewe



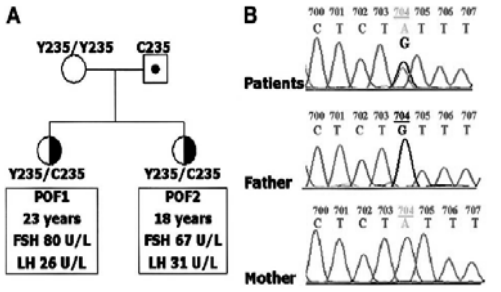
Galloway, Nat Genet 2000; 25 : 279-283
Shimasaki S Endocr Rev 2004; 25 : 72



2 sisters with POF

Streak ovaries

Di Pasquale Am J Hum Genet 2004; 75



Xp 11.2

Di Pasquale Am J Hum Genet 2004; 75

N= 203 POF patients

Gene	Sequence variation	AA change
BMP15	788insTCT	Ins263L
	443T > C	L148P
	538G > A	A180T
	852C > T	S284S
	831T > C	T277T
	468G > A	V156V

SA

Laissue P, EJE 2006, 154: 1-7

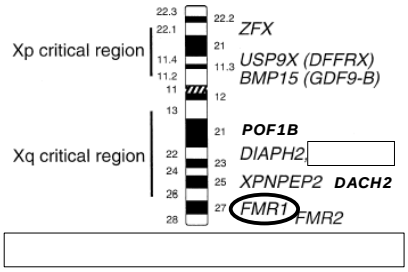
N=166 POF women variants 9/166

Patient no.	Gene variation	Protein variation	Phenotype
36	c. 538G>A	p. A180T	SA
73	c. 202C>T	p. R68W	SA
79	c. 538G>A	p. A180T	SA
92	c. 538G>A	p. A180T	SA
112	c. 538G>A	p. A180T	SA
113	c. 538G>A	p. A180T	SA
118	c.704 A>G	p. Y235C	PA
124	c.788insTCT	p.262insLeu	PA
125	c.788insTCT	p.262insLeu	SA

Di Pasquale J Clin Endocrinol Metab 2006

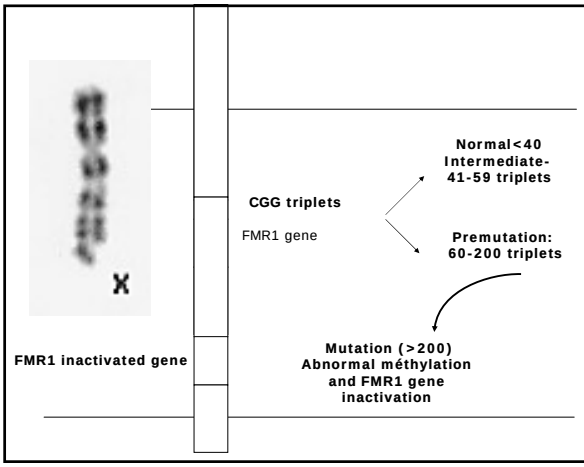
- BMP15 polymorphisms or mutations?
- *In vitro* studies are necessary

X Chromosome



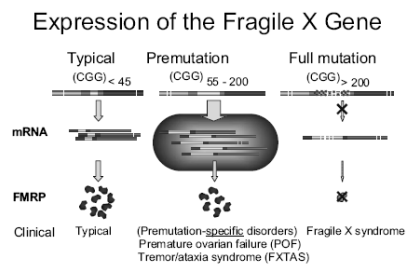
FMR1 gene

- X fragile syndrome
- 1st cause of mental retardation in boys
- CGG triplets 5'UTR region



N<41
intermediary 41-59
premutated 60-200 (1:590 women)
mutated > 200

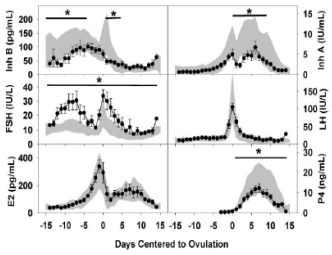
Expression of *FMR1* in normal women, premutation carriers, and full mutation carriers. Figure adapted from Hagerman and Hagerman (10).



Wittenberger. FMR1 premutation. Fertil Steril 2007.

Premutated women

N=11



C Welt 2004 JCEM 80 : 4969

20% of women with a premutation have POF

Sherman Am J Med Genet 2000; 97: 189

A premutation is present in 3% of sporadic POF cases
A premutation is present in 13% of familial POF cases

Conway G Hum Reprod 1998; 13: 1184
Sullivan AK Hum Reprod 2005; 20: 402

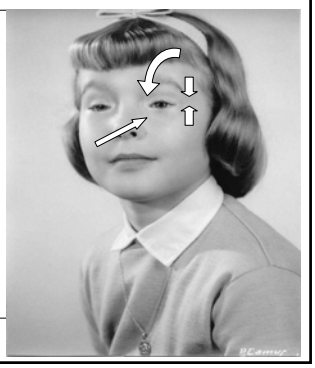
No linear correlation between the number of triplets and POF

Highest risk if 80-100 triplets

Sullivan AK Hum Reprod 2005; 20: 402
Ennis S Eur J Hum Genet 2006; 14: 253

1. BPES: definition

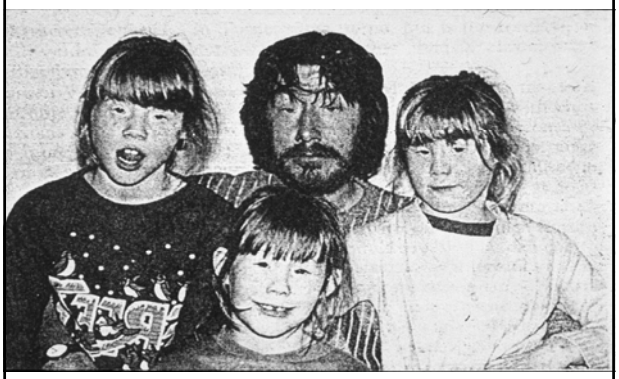
- Blepharophimosis
- Ptosis
- Epicantus inversus



Syndrome de BPES
Blepharophimosis, Ptosis, Epicanthus inversus Syndrome

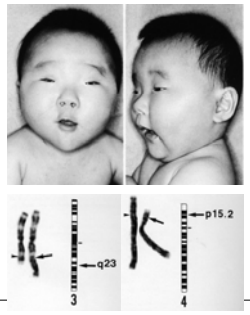
- **Type I : POF and eyelids abnormalities**
  - **No testicular phenotype**
- **Type II : Eyelid abnormalities, No Gonadal Phenotype**

Blepharophimosis Epicanthus Syndrome



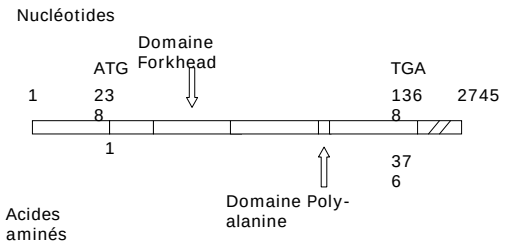
BALANCED TRANSLOCATION

- translocation patient t(3;4) (q23; p15.2)



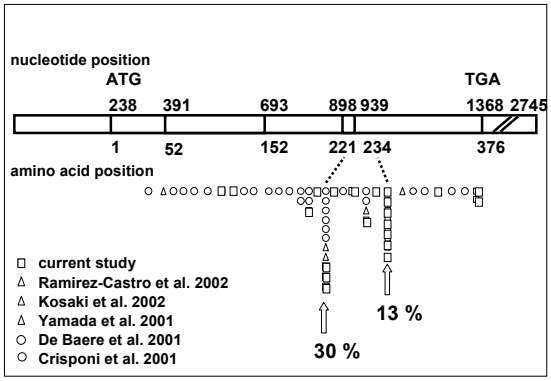
Fukushima *et al*., *Am J Med Genet*, 1991.

FOXL2

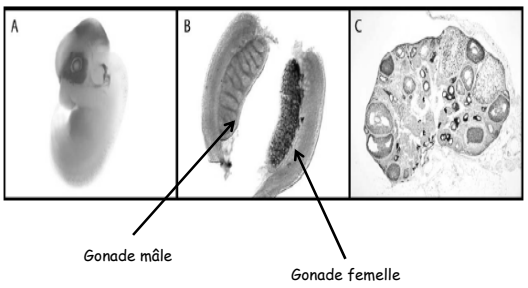


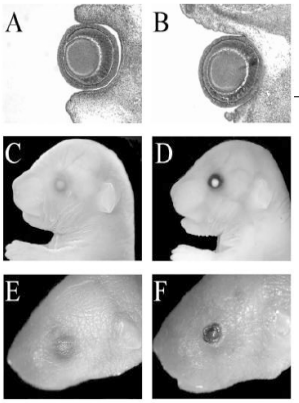
d'après *De Baere et al*., *2001*

FOXL2: MUTATIONAL HOTSPOTS



Marquage Foxl2

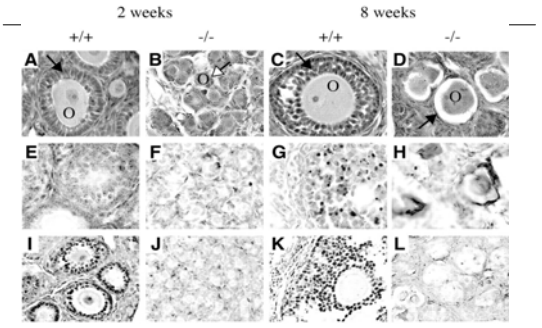




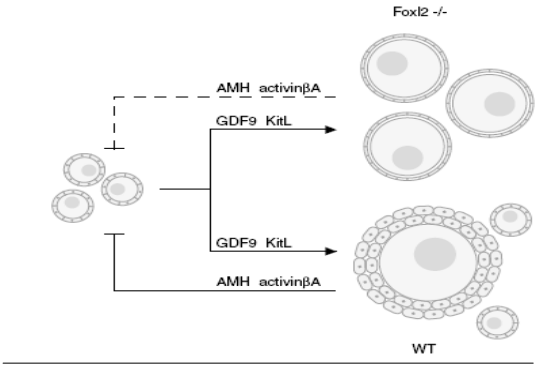
Eyelid anomalies.
(Uda et al)

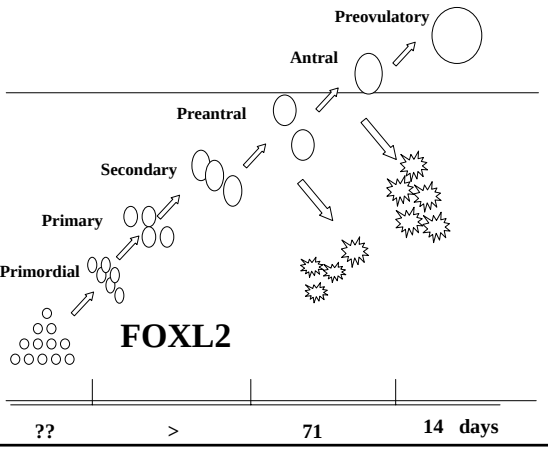
Defects are apparent in 13.5 dpc *Foxl2-/-* embryos (B) by histology with hematoxylineosin staining, correlated with overt eyelid hypoplasia and open eyes at 17.5 dpc (D) and birth (F) compared to wild-type animals (A, C, and E).

FOXL2 -/- mice model



Meier Devlopment 2004

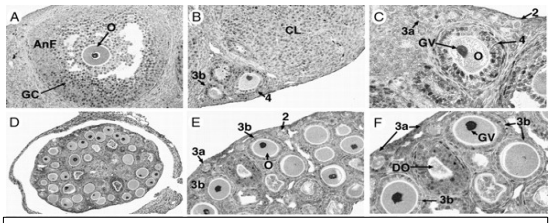




FOXL2: gene in sporadic POF ?

- 100 POF patients without BPES: no mutation
  De Baere et al., 2002
- 70 POF patients without BPES : 2 mutations ?
  Harris et al., 2002
- 120 POF patients without BPES : no mutation
  Bodega et al., 2004

2-GDF9 : growth differenciation factor 9



GDF9 -/- mouse model

Elvin Mol Endocrinol 1999; 13 :1018

GDF9

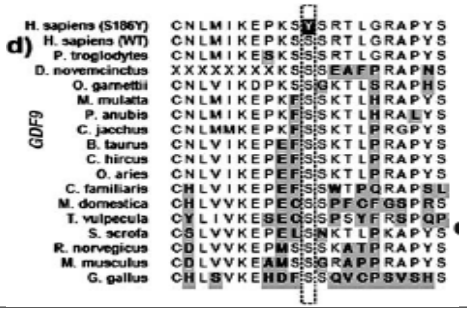
- ☐ 127 POF women
- ☐ 220 controls
- ☐ 8 variants : mutations ou polymorphisms?

Dixit H Menopause, 2005

N=203

GDF9	557C > A	S186Y	→	Menarche 9,5 Regular menstrual cycles 21 days Amenorrhea 33 FSH = 104 mUI/L Biopsy : absence of follicles
	447C > T	T149T		
	546G > A	E182E		

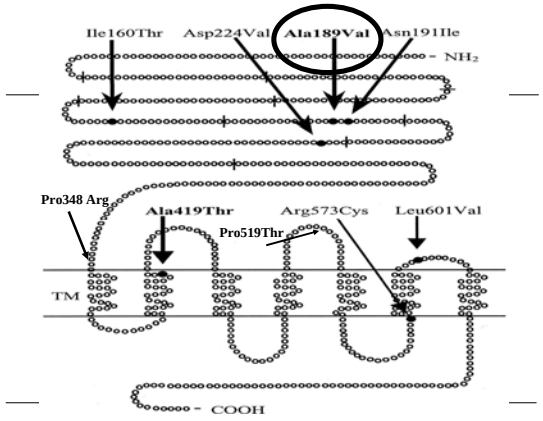
EJE 2006, 154: 1-7



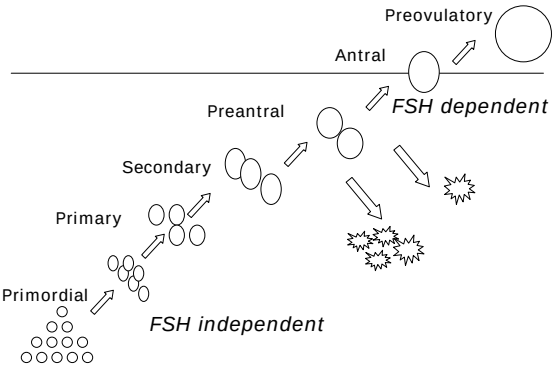
3-FSH receptor

- Families with > 3 cases of primary amenorrhea
- North of Finland
- Linkage analysis :
  chromosome 2p

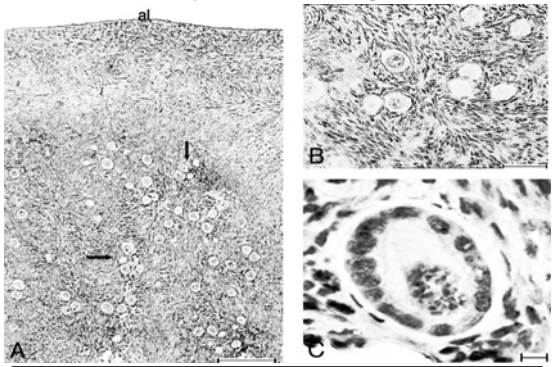
Aittomaki K, Cell 1995; 82: 959



Doherty, J Clin Endocrinol Metab 2002; 87: 1151



FSH receptor inactivating mutations



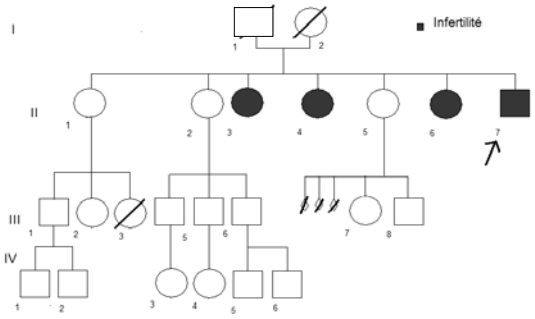
G Méduri J Clin Endocrinol Metab 2003; 88 : 3491

Mr R, 34 , Infertility

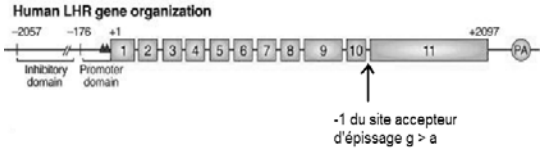
- puberty: 13-14
- 88 kg, 165 cms: BMI 32kg/m2
- Testes volume 20-25 mL
- Reduced penis size
- P2-P3, Shaving x 1 / month
- Spermogram :
  - Small Volume : 0.35 et 0.5 ml
  - 3.2 millions sp / ejaculate
  - Low vitality
  (20 et 14%)

- Hormonal Assessment:
  - Testosterone: 0.9 nmol/L (N: 11-40)
  - LH: 30 U/L (N: 0.5-11)
  - FSH: 10.3 U/L (N: 0.8-13)
  - Inhibin B: 153 ng/mL (N: 135-350)
  - AMH: 222 pmol/L (N: 26-100)
  - PRL: 19.5 ng/L, E2: < 5 pg/mL
- Testicular US:
  Right T: 49 x 28 mm, Left T: 50x30 mm.

- Caryotype 46 XY (170 cellules)
- Negative hCG Test :
  - Testostérone: from 2.8nmol/L to 2.2 nmol/L @ 96h
  - LH: 17 U/L , FSH: 9 U/L



Résultats (I):

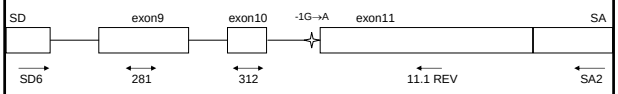


homozygote mutation g → a en –1 du site accepteur d'épissage de l'intron 10 avant le début de l'exon 11.

RESULTS

1- The intron 10 acceptor splice site appeared to be an inefficient site. We had to cut out certain sequences from exon 111, since they were used as preferred splice acceptors for exon 10.
2- The mutation makes the acceptor site even worse: less product of the right size AND another splice product is found: **24 bp downstream another splice site is used, that results in a deletion of the first 8 AA of exon 11!** This does not happen in the wt.

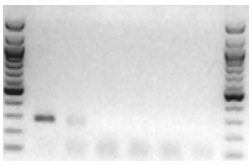
So: the mutations causes less LH receptor to be produced + the mutations causes a different LH receptor protein to be produced.



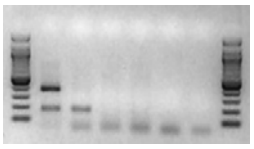
Splice vector based on pSPL3. Numbers below the primers.

Splice products of exon9 +10 +11 (intron10 -1 G→A), ΔXba/BgI, no vector SA site (duplo)

Lane order in all gels:
1 - marker
2 - wt
3 - mutant
4 - empty vector
5 - noRT reaction
6 - no RNA reaction (H2O)
7 - plasmid control



Primers 312FOR + 11.1REV (282)



Primers 281FOR + 11.1REV (383)

Above: less splice product of the right size (282) in the mutant + a slightly smaller product - we have sequenced that product: 8 AA missing from the start of exon 11, but in frame!
See also next slide, different experiment - same result, but more efficient splicing.

Left: when including a primer in exon 0: even less product in the mutant.

Résultat (II): Etude familiale

Sujet index et 2 sœurs homozygotes,
1 sœur hétérozygote
pour la mutation de RLH

Mrs EF (II6), 42 homozygote

- Menarche : 16 ans,
- Regular cycles (28 days) until age 20 ans followed by oligomenorrhea (2 -6 months).
- At age 30 CC treatment
  => follicular development up to 10, 12, et 15mm, with no endometrial growth beyond 8 mm
  => No d'ovulation : progesterone= 0.6ng/ml.

Mme EF (II6), 42 ans, homozygote

- Two IVF attempts:

  => multifollicular develoment (14 follicles , 9-14mm & 18 follicles, 9-25 mm) after Rec FSH
  =>estradiol: 910 et 1260 nmol/L
  => Following hCG , No Oocyte retrieval on 2 occasions.

- Progestin induced menstruation

- After treatment cessation: at age 42, : persistance of irregular spontaneous menses

  On day 2: E2: 138 pg /mL, LH: 9 U/L, FSH: 9.5 U/L.

Mrs JF (II3), 53, homozygote

- Menarche: 16-17
- Oligomenorrhea 90 to 240 day cycles
- Failure of several attempts of ovarian stimulation
- Menopause @ 51 ans

Mrs MD (II5), 44, heterozygote:

- Menarche: 13-14
- Regular cycles,
- G5P2,

Table II. Autoimmune polyglandular syndromes and POF

APS type	Inheritance	Autoimmune involvement	Age group	Incidence of POF
APS I	Autosomal recessive caused by a mutation in the autoimmune regulator (*AIRE*) gene on chromosome 21	Chronic mucocutaneous candidiasis, adrenal and parathyroid failure	Children age 3–5 years or in early adolescence	17–50% (Ahonen *et al.*, 1990)
APS II (more common)	Polygenic, characterized by dominant inheritance and association with HLA DR3	Primary adrenal failure (Addison's disease) with autoimmune thyroid disease (Schmidt's syndrome) and/or type 1 diabetes (Carpenter's syndrome)	Adults in the third or fourth decade	3.6–10% (Betterle *et al.*, 2004)
APS III	Apart from the absence of adrenal failure, no clinical differences between types II and III have been described	Thyroid failure and other immunological syndromes with exclusion of Addison's disease	Adults	

GENES IDENTIFIED IN VERY FEW CASES

In 2008 many candidate genes

TABLE 1. Established Mendelian disorders (and OMIM number) with ovarian failure as one component[a]

Ataxia–telangiectasia (#208900)
Bloom syndrome (#210900)
Cockayne syndrome (#216400)
Martsolf syndrome (#212720)
Nijmegen syndrome (#251260)
Rothmund–Thomson syndrome (#268400)
Werner syndrome (#277700)

POF

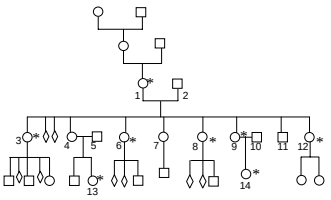
- Autosomes
  - ***FSHR***
  - ***FOXL2***
  - ***GDF9***
  - ***ATM***
  - ***AIRE***
  - ***NOBOX***
  - ***GALT***
  - ***EIF2B***
  - ***NSB1***
  - ***DMC1***
  - **Parathyroid responsive B1 gene**
  - ***FIGLA***
  - ***Progesterone receptor membrane component-1 (PGRMC1)***
- X linked
  - **X Monosomy**
  - **X,XX mosaicism**
  - **X ring**
  - **Triple X**
  - **X Deletions**
  - **X, autosome translocations**
  - ***FMR1***
  - ***BMP15 ……..***

Perspectives

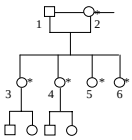
- Genome scanning of familial cases

Families IOP - France

Famille FR1DA



Famille FR2BO



○ Cas IOP
* ADN disponible

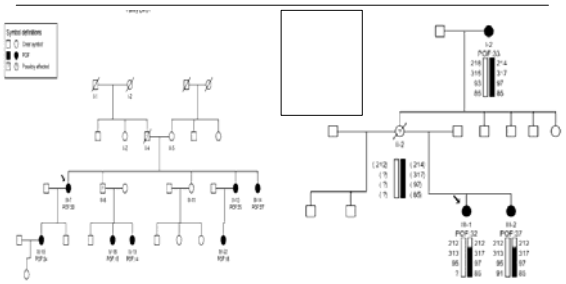
Human Reproduction pp. 1–7, 2008 Hum. Reprod. Advance Access published August 9, 2008 doi:10.1093/humrep/den278

A genome-wide linkage scan in a Dutch family identifies a premature ovarian failure susceptibility locus

R.A. Oldenburg[1], M.F. van Dooren[1], B. de Graaf[1], E. Simons[1], L. Govaerts[1], S. Swagemakers[2], J.M.H. Verkerk[2], B.A. Oostra[1] and A.M. Bertoli-Avella[1,3]

[1]*Department of Clinical Genetics, Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands;* [2]*Department of Bioinformatics, Erasmus Medical Center, Rotterdam, the Netherlands*

region on chromosome 5q14.1–q15



Perspectives

- ☐ Genome scanning of familial cases
- ☐ Genome scanning of sporadic cases

- ☐ Candidate genes from animal models?

CONCLUSIONS

POF 1-2% of women
Xfra premutation ++
Familial cases++
Hope in genome wide studies

GENES of MENOPAUSE

New Contraceptive Targets

New Infertility Treatment

D Dewailly, AC Reyss
R Frydman, L Jacquesson
P Bouchard, S Christin- Maître, N Bourcigaux,
B Donadille, C Dupas

MF Portnoï, L Finkel, JP Siffroi
G Tachdjian, A Aboura, G Rousseau

M Fellous, R Veitia, P Laissue
M Lathrop, D Zelenika, S Heath